REMARKS

Claims 1 and 3-16 are pending. Claims 13-16 have been withdrawn from consideration.

35 U.S.C. 103 obviousness rejection – Secondary considerations

The examiner has rejected the current claims under § 103 as being obvious over a combination of prior art references. While applicant contends that the combination of references cited by the examiner do not in fact support a § 103 rejection for the reasons discussed below, more to the point is the fact that, powerful secondary considerations are extant that strongly militate against a finding of obviousness. These secondary considerations include, without limitation, a long-felt, unfulfilled need for the invention and the express failure of others to fill that need. That is, the examiner is directed to and requested to consider the following:

1. Guttman, D., et al., "Multiple Infections of *Ixodes sapularis* Ticks by *Borrelia burgdorferi* as Revealed by Single-Strand Conformation Polymorphism Analysis," J. Clinical Microbiology, **1996**, 652-656. In this paper, ticks infected with four strains of *B. burgdorferi*, arbitrarily designated mobility classes 1 – 4, we analyzed. On page 654 of the Results, the authors state that "From the DNA sequencing, there is no way to show that MC3 is in the sample when both MC1 and MC4 are present."

Thus we have here an early indication of a need or desire to be able to identify multiple infective strains in an individual by DNA sequencing and an express statement of failure to fulfill that need, the failure further demonstrating the difficulty in doing so, which further suggests that at the time, 1996, those skilled in the art would have been very skeptical that DNA sequencing could achieve the desired goal.

2. Jacobs, M. V., et al., "Reliable high risk HPV DNA testing by polymerase chain reaction: an intermethod and intramethod comparison," J. Clin. Pathology, **1999**, 52:498-503. In this reference, humanpapillomaviruses, HPVs,

is specifically addressed. On page 502, under "Intermethod comparison" it is stated that

> Direct sequencing of GP5+/6+ PCR products apparently failed to identify under-represented HPV types in the multiple HPV infections"

once again at last impliedly indicating that a need or desire to identify distinct strains of HPV existed and that the need was unmet; specifically by use of DNA sequencing.

3. Gharizadeh, B., et al., "Typing of Human Papillomavirus by Pyrosequencing," Laboratory Investigations, **2001**, 81(5) 673-79. In this paper, pyrosequencing, which at the time was a relatively new technique (Ronaghi, et al, 1998), was for the first time compared to conventional DNA sequencing techniques to genotype HPVs. The same GP5+/6+ primer previously used by Jacobs, et al., was employed. On page 677 of the report, the authors stated:

> As with other available methods, multiple infections present in one specimen might be problematic to detect, depending on the proportional dominance and number of genotypes present in the amplicon. At present, pyrosequencing might not be particularly useful for identifying infection with more than one HPV genotype because multiple infections give sequence signals from all of the available types on the specimen. Typing may be possible provided one type is solidly dominant, with low background signal(s) from other existing genotypes. However, this information alone may be insufficient.

Here, then is another implicit indication of the desirability to be able to detect multiple infections in one subject and an express statement that the authors – and therefore those skilled in the art - doubted that such could be accomplished by DNA pyrosequencing.

4. van Doorn, L-J, et al., "Molecular detection and genotyping of human papillomavirus," Expert Rev. Mol. Diag., **2001**, 1(4):394-402. This review, published two years prior to the filing date of the current application corroborates Gharizadeh's position:

> Rapid sequencing methods are now becoming available for high-throughput to permit application in routine analysis of clinical samples. However, it should be noted that sequence analysis is not very sensitive to simultaneously detect different sequences in a mixture. Sequences only representing a minority of the total PCR product may easily remain unnoticed and only the predominant genotype will be detected. This may be insufficient to analyze clinical samples containing a mixture of different HPV genotypes and will underestimate the prevalence of infections with multiple HPV genotypes, which has important consequences... (Page 397.)
>
> The presence of multiple HPV genotypes is a common phenomenon in some patient groups. Up to 35% of HPV-positive samples form patients with advanced cytologic disorders and more than 50% of HIV-infected patients contain multiple HPV genotypes... (Page 397.)

This review unambiguously states the problem, implies that a need exists to solve it given the percentage of patients so infected, and further expressly notes that it could not be solved using DNA sequencing.

5. Wall, S. R., "Cervical human papillomavirus infection and squamous intraepithelial lesions in rural Gambia, West Africa: viral sequence analysis and epidemiology," British, J. Cancer, **2005**, 93:1068-76. In this paper, which, significantly, was published at least two years after the 2003 filing date of the present application, the need to be able to separately determine strains of HPV is once again expressly set forth:

> HPV DNA sequence must therefore be defined to ensure vaccine efficacy and present selective emergence of rare, virulent variants." Page 1068.

On page 1070, however, the authors state:

> These samples were sequenced with the MY 09/11 primers; five had either degenerated in storage or would not amplify, two contained multiple templates and were impossible to sequence [exactly what the current invention is directed to] ... (emphasis added).

Thus, in 2005, the need was still present, it was still unmet and DNA sequencing was becoming less and less appealing as a means of solving the problem to those skilled in the art.

6. Giuliani, L., et al., "Comparison of DNA sequencing and Roche Linear Array® in human papillomavirus (HPV) genotyping," Anticancer Research, **2006**, 26:3939-41. This article once again emphasizes that DNA sequencing was considered at the time, three years after the filing of the current invention, as not being useful for determination of multiple infections:

> Conclusion: The Roche Linear array® [a non-sequencing technique] is a highly accurate assay for HP genotyping. This is particularly true in the presence of multiple infections **which DNA sequencing is unable to resolve**. (Emphasis added.)

7. Lee, S. H., et al., "Routine human papillomavirus genotyping by DNA sequencing in community hospital laboratories," Infectious Agents and Cancer, **2007**, 2:11. Here, 6 years after the filing date of the current application, a DNA sequencing method capable of determining multiple infections in a single reaction still constituted a highly desirable and diligently sought after need that as of this time was considered unmet:

> Among the 107 nested PCR-positive samples, DNA sequencing with the GP6+ consensus general primer yielded multiple overlapping unreadable sequences in 5 cases. Using the individual type-specific primer sequencing for HPV-6 , -11, -16 and -18 proved that one of them contained HPV-16, but not the other three genotypes, and that one contained a mixture of HPV-16 and HPV-18, but not the other two genotypes. For the remaining 3 mixed infection samples, repeated individual DNA sequencing failed to produce a readable primer extension/termination reaction with any of the four type-specific primers. Therefore, these 3 latter cases were considered to be multiple infections caused by HPVs other than the four vaccine-relevant types and grouped under the 'low-risk" category. (Page 6 of 11, emphasis added.)

It is simple beyond question that as recently as one year ago there was no DNA sequencing technique, pyrosequencing or otherwise, that was capable of determining multiple infections. The above citations establish that the need for such a technique was felt for at least 10 years and was clearly unmet. It further is evident that numerous groups of skilled artisans had tried to find such a sequencing technique and had failed. Thus, the secondary considerations alone militate strongly against any assertion that the present technique would be found obvious to those skilled in the art. In fact, it is also fair to say that the abject failure of so many to find a DNA sequencing technique that could simultaneously identify multiple infective agents in a single subject was so pervasive that the art was unquestionable teaching away from the current invention, i.e., it was say in effect, stay away DNA sequencing will not work.

The examiner is requested to reconsider and withdraw the rejections based on the above secondary considerations alone. The following is presented to point out that, secondary considerations aside, the art cited by the examiner does not in any event render the current invention obvious.

35 U.S.C. § 103 Rejection of claims 1, 3-6 and 17

The examiner rejected claims 1, 3-6 and 17 as being unpatentable over Alderborn, et al., Genome Res., **2000**, 10(8):1249-58, in view of Ronaghi, Anal. Biochem., **2000**, 286(2):282-8 and, further, in view of Caskey, et al., U.S. Pat. No. 5,582,989.

In the examiner view, Alderborn teaches determination of single-nucleotide polymorphisms (SNPs) using real-time pyrophosphate DNA sequencing by providing sample containing a target DNA with variable sequence regions, amplifying the DNA, providing sequencing primers, hybridizing the primers to the target DNA and sequencing the variable regions by pyrosequencing.

The examiner admits that Alderborn does not teach using a mixed pool of structurally different primers, each primer being specific for one species, group or target and mixing such mixed pool of primers with a target DNA. The examiner notes, however, that Alderborn teaches identification of multiple SNPs on a

single target DNA using one primer and then opines that this makes it clear in the art that multiple SNPs could be resolved in one reaction.

The examiner next opines that, insofar as using two structurally different primers to resolve multiple SNPs sites is concerned, Ronaghi teaches that even after addition of single-stranded binding protein to a pyrosequencing procedure, the read-length is limited to about 30 nucleotides.

The examiner also states that the extension of multiple primers, each primer drawn to a different target sequence in the same reaction, which the examiner characterizes as multiplex extension, was a well-known concept at the time the current invention was filed and then cites Caskey as highlighting the advantages of multiplexing nucleic acid amplification using structurally different primers, including the ability to detect multiple different separated target sequences as well as the ability to dectect multiple different loci of the same target sequence separated by large sequences in between the loci.

Specifically as to claims 3 and 4, the examiner opines that Caskey teaches multiple different target DNA from virus and bacteria.

Likewise, with regard specifically to claims 5 and 6, the examiner opines that Caskey teaches multiple different disease-linked variants.

Based on the above, the examiner concludes that it was clear at the time of the invention that multiple different target sequences could be extended in a polymerase extension reaction and that it therefore would have been prima facie obvious to a skilled artisan at the time of the invention to use multiple structurally different primers within the same reaction for detection of multiple different SNPs separated by large sequences, i.e., multiplex SNP detection, since the prior art demonstrates that such primers can extend different target loci separated by large sequences and that the skilled artisan would have a reasonable expectation of success since the prior art demonstrates that pyrosequencing can identify multiple different SNP sites within the same reaction.

Applicant traverses.

Applicant's response

First it is noted that Alderborn has nothing whatsoever to do with identifying multiple infections by different species in an individual. All Alderborn did was compare two alleles of the same gene to determine whether one of them contained one or more single nucleotide polymorphisms , which he determined by using a simplistic pattern recognition procedure. That is, the patterns of the two alleles would be more alike than different, differing only if a SNP was present. If the genes had been derived from two different species, it is evident that Alderborn's pattern recognition would be incapable of reveal anything. Furher, as the examiner freely admits, Alderborn does not teach using a mixed pool of structurally different primers, each primer being specific for one species, group or target and mixing such mixed pool of primers with a target DNA. Neither, however, does Caskey. Caskey does indeed use more than one primer; however, as the examiner notes, Caskey is performing a standard multiplex PCR reaction as described initially by Chambelin, et al. in 1988. That is, multiplex PCR as practiced by Caskey does indeed enable simultaneous amplification of many targets of interest in one reaction using more than one pair of primers. The targets of interest, however, comprise different locales on one and the same strand of DNA to educe multiple deletions, mutations and/or polymorphisms in that particular DNA. Multiplex PCR as initially described and as practiced prior to the present invention simply could not be used to identify multiple different strains in a single sample as the references cited above clearly indicate. That is, amplification and extension is not synonymous identification due to probably biases, which is why multiplex PCR is most often combined with other techniques for accurate identification.

The examiner is requested to reconsider and thereupon withdraw the rejection.

35 U.S.C. § 103 rejection of claims 7-12

The examiner has rejected claims 7-12 under § 103(a) as being unpatentable over Aldeborn, et al., in view of Ronaghi, Caskey, et al. and, further In view of Rader, et al., "Type-specific primer-mediated direct sequencing of

consensus primer-generated PCR amplicons of human papillomavirus: a new approach for the simultaneous detection of multiple viral type infections," J. Virol. Methods, **1995**, 53(2-3):245-54. The examiner refers back to the arguments posed in the above rejection insofar as Alderborn, Ronaghi and Caskey are concerned but then notes that none of those articles specifically teach the sequencing of HPV. The examiner then notes that Rady teaches amplification of a conserved region within multiple different HPV types and subsequent sequencing with sequence-specific primers, materials and methods. That examiner states that, with regard to claim 11, sequencing of low yield amplification of fragments is inherent to the methods of Ye. Then, the examiner argues that would have been *prima facie* obvious to a skilled artisan at the time of the current invention to use the methods suggested by the prior applied references to detect certain HPV types within a sample since the prior art demonstrates such methods are capable of identifying many different nucleic acid templates within the same reaction. Finally, with regard to claim 12, the examiner argues that Caskey teaches the importance of designing primers that do not anneal to unspecified sequences and that it would therefore have been obvious to the skilled artisan at the time of the invention to design multiplex sequencing primers such that they do not anneal to unspecified sequences and thereby provide erroneous sequencing information.

Applicant traverses.

Applicant's response

As discussed above, at the very most, all that Alderborn, Ronaghi and Caskey teach up to the time of the present invention is multiplex PCR to reveal multiple deletions, insertions, mutations and/or polymorphisms in a single strand of nucleic acid taken from a single gene. There is nothing in their disclosures to even remotely render the present invention obvious. Rady does nothing to remedy the situation. All that Rady teaches is HPV amplification with consensus and degenerate GP and MY primers and then sequencing the amplicons with the general primer by Sanger dideoxy sequencing. Rady does not use any multiple specific sequencing primers and does not, and cannot, detect multiple infections.

The PCR cannot differentiate between different HPV types and the described DNA sequencing cannot differentiate between multiple HPV infections. There is nothing in Rady to suggest otherwise.

The examiner is requested to reconsider and thereupon withdraw the rejection.

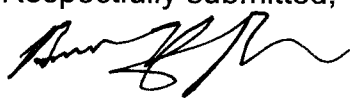
CONCLUSION

Based on the above remarks, in particular the secondary considerations discussed above and which virtually eliminate any possibility of obviousness, applicant believes that this application is in condition for allowance and respectfully requests that it be passed to issue.

A one month extension of the time for filing this response is requested and the Commissioner is authorized to charge the fee due to Squire, Sanders & Dempsey Deposit Account No. 07-1850.

Date: 25 September, 2008

Respectfully submitted,



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